Water Vapor Permeability of Mammalian and Fish Gelatin Films

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ABSTRACT: Water vapor permeability of cold- and warm-water fish skin gelatins films was evaluated and compared with different types of mammalian gelatins. Alaskan pollock and salmon gelatins were extracted from frozen skins, others were obtained from commercial sources. Water vapor permeability of gelatin films was determined considering differences on percent relative humidity (%RH) at the film underside. Molecular weight distribution, amino acid composition, gel strength, viscoelastic properties, pH, and clarity were also determined for each gelatin. Water vapor permeability of cold-water fish gelatin films (0.93 gmm/m^{2}hkPa) was significantly lower than warm-water fish and mammalian gelatin films (1.31 and 1.88 gmm/m^{2}hkPa, respectively) at 25 °C, 0/80 %RH through 0.05-mm thickness films. This was related to increased hydrophobicity due to reduced amounts of proline and hydroxyproline in cold-water fish gelatins. As expected, gel strength and gel setting temperatures were lower for cold-water fish gelatin than either warm-water fish gelatins or mammalian gelatins. This study demonstrated significant differences in physical, chemical, and rheological properties between mammalian and fish gelatins. Lower water vapor permeability of fish gelatin films can be useful particularly for applications related to reducing water loss from encapsulated drugs and refrigerated or frozen food systems.

Keywords: water vapor permeability, fish skin, gelatin, edible films

Introduction

Gelatin versatility and functionality reflects the fact that it is the only food protein that undergoes a thermally reversible helix-coil partial transition to resemble its original parent protein structure, collagen (Stainby 1977). As gelatin solutions age, water is expelled from the linear and flexible portions of the protein network, which then collapses into a rubbery film that vitrifies with hydrogen bonding cross-links upon drying. A typical fringed micelle, self-limited in size structure, is originated as regions of extended parallel polypeptide chains and are not closely bound at ends (Lukasik and Ludescher 2005).

The mechanical properties of films made from gelatin gels cured above or below the triple helix melting temperature differ, with hot-cast films typically being more brittle than cold-cast films. The mechanical and barrier properties of these gelatin films are modulated by additives such as polyols and large molecular weight carbohydrates (Lukasik and Ludescher 2005). It is expected that gelatins, as other protein films, have sufficiently low oxygen permeability to serve as effective barriers, especially at low water activities (Krochta and De Mulder-Johnston 1997; Krochta 1998; Sothornvit and Krochta 2000; Perez-Gago and Krochta 2001; Carvalho and Grosso 2004). Water vapor permeability of gelatin and other protein films increases linearly with increasing concentration of hygroscopic plasticizers such as sorbitol and glycerol (McHugh and others 1994; Sobral and others 2001).

Gelatin from marine sources (warm- and cold-water fish skins, bones, and fins), which has not been previously explored, is a possible alternative to bovine gelatin. One advantage of marine gelatin sources is that they are without the risk associated with Bovine Spongiform Encephalopathy outbreaks. In addition, they are Kosher and Halal. Potential sources of gelatin that have been underutilized include the skins from Alaskan pollock (*Theragra chalcogramma*) and Alaskan pink salmon (*Oncorhynchus gorbuscha*). These two species comprised approximately 73% of the annual marine finfish catch of Alaska in 2000 (Crapo and Bechtel 2003). It has been estimated that over a million tons of fish byproducts are generated each year from the fishing industry in Alaska (Crapo and Bechtel 2003). Some of these byproducts are converted into fishmeal; however, approximately 60% are dumped back into the ocean (Crapo and Bechtel 2003). These byproducts include fish skin, which is a good source of gelatin. Cold-water fish gelatins, such as those extracted from pollock and salmon, have very low gelation and melting temperatures compared to mammalian and warm-water fish gelatins. This is due to the cold-water fishes having lower concentrations of proline and hydroxyproline than the other species (Haug and others 2004). The amino acid compositions of mammalian gelatins are remarkably constant when compared to those from different species of fish. Glycine, the simplest amino acid, accounts for approximately one-third of the total amino acid residues in mammalian gelatins, proline and hydroxyproline for approximately one-fifth, and alanine for approximately one-ninth. In all, these four amino acids account for approximately two out of every three amino acid residues in mammalian collagen used in gelatin manufacturing (Balian and Bowes 1977). Fish collagens show a wider variation in composition. Their hydroxyproline and, to a lesser extent, proline contents are lower than that of mammalian collagens and this is compensated for by higher concentrations of serine and threonine (Balian and Bowes 1977). Consequently, cold-water fish gelatins behave as a viscous liquid at room temperature, which limits their use in many applications. To our knowledge there exists no published research on water vapor permeability of fish gelatins despite their excellent film-forming properties with potential high barrier efficiency that can have applications for edible films and coatings.

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The objective of this study was to evaluate the water vapor permeability properties of different types of mammalian and warm- and cold-water fish skin gelatin films and to correlate these results to protein molecular weight distributions, amino acid compositions, and gel properties.

Materials and Methods

Fish skin gelatin extraction

Skins of fresh Alaskan pollock (*Theragra chalcogramma*) were obtained from Western Alaska Fisheries processing plant in Kodiak, AK, U.S.A. The skins were mechanically removed from fillets and immediately collected, drained, frozen, shipped, and stored at −20 °C for 4–6 mo at the Western Regional Research Center (WRRC), Albany, Calif., U.S.A. Whole Alaskan pink salmon (*Oncorhynchus gorbuscha*) were collected from Western Alaska Fisheries processing plant, immediately frozen and shipped to the WRRC, where the skins were manually separated and stored at −20 °C for up to 3 mo.

Thawed strips of skins (0.68 kg) were washed by mixing with 3.8 L of iced water (1:6 w/v) for 10 min at 2 °C to 5 °C in a stainless steel bucket with a paddle mixer at 70% speed capacity (Groen/Dover Ind., Jackson, Mich., U.S.A.) and then rinsed with iced tap water. Excess water was removed by draining the cleaned skins in a stainless steel sieve for 5 min and manually squeezing skins in the sieve. This washing step was repeated three times. Skins were further cleaned for an additional three times using the same procedure with cold (5 °C) 0.8 N NaCl (1:6 w/v) for muscle protein solubilization.

Washed skins were stirred with cold 0.2 N NaOH (1:6 w/v) for 40 min for fish skin swelling and loss of amide-nitrogen, drained, and then stirred with 0.2 N sulfuric acid using the same conditions. This procedure was repeated three times. Samples were drained and rinsed with iced tap water after each step. Skins were then treated with 0.7% citric acid (1:6 w/v) for 40 min with continuous stirring (repeated three times with draining and rinsing with iced tap water after each step) and then partially dissolved overnight with distilled water in a water bath at 45 °C without stirring according to Montero and Gomez-Guillen (2000) and Gudmundsson and Hafsteinsson (1997). These dilute acid treatments lead to collagen solubilization at temperatures above 20 °C due to the action of acids on labile intermolecular links of the Schiff's base type (Balian and Bowes 1977). Fish scales were separated from the skin by the consecutive washing and rinsing steps with the different acid and alkaline solutions.

This mixture was filtered under vacuum in a Büchner funnel with Whatman nr 4, 110-mm dia, filter paper and the clear filtrate was then air dried in a convection oven at 45 °C until moisture content was less than 15%. Yield was estimated at 13% (weight of dried gelatin/weight of thawed fish skin).

Lab-extracted cold-water fish skin gelatins from pollock and salmon were compared with three commercial cold-water fish skin gelatins derived from pollock, cod or haddock, a warm-water fish catfish skin gelatin, and five 250 Bloom mammalian gelatins. Table 1 lists short notations and the different specific types of studied gelatins.

Table 1—Cold-water, warm-water fish skin and mammalian gelatins studied

Gelatin type	Short notation
Lab extracted from frozen pollock (cold-water fish) skin	Pollock
Lab extracted from frozen salmon (cold-water fish) skin	Salmon
Commercial high molecular weight cold-water fish skin	HMW CWF
Commercial cold-water hydrolyzed[a] fish skin	HYD CWF
Commercial cold-water fish skin	CWF
Commercial (warm-water fish) catfish skin	WWF Catfish
Commercial brand 1, type A[b] pork skin	Pork skin 1
Commercial brand 2, type A pork skin	Pork skin 2
Commercial brand 2, type B[c] cattle hide	Cattle hide
Commercial brand 3, type B non-deionized[d] cattle bone	ND cattle bone
Commercial brand 3, type B deionized[e] cattle bone	D cattle bone

[a]Collagen protein chains highly broken down into small chain peptides.
[b]Produced via acid processes.
[c]Produced via alkaline processes.
[d]Contains mineral salts derived from processing.
[e]Contains only trace amounts of mineral salts.

Molecular weight distribution

Gel electrophoresis (SDS-PAGE) samples were prepared by adding dried gelatin to deionized water at a concentration of 67 mg/mL. The gelatin was dissolved in deionized water by heating the sample in a 40 °C water bath for 30 min. The sample was then centrifuged for 5 min at 20000 *g* using an Eppendorf 5415 centrifuge (Eppendorf AG, Hamburg, Germany). The supernatant was diluted with deionized water to 400 μg/mL, denatured with NuPAGE® LDS Sample Buffer (Invitrogen Life Technologies, Carlsbad, Calif., U.S.A.), and reduced with dithiothreitol (Invitrogen Life Technologies) in a 70 °C water bath for 10 min. Electrophoresis was then performed by adding 2 μg gelatin sample in each lane of a 3% to 8% NuPAGE® Tris-Acetate gel with Tris-Acetate SDS running buffer in an XCell SureLock™ electrophoresis unit (Invitrogen Life Technologies). Reference marker was HiMark™ Unstained Protein Standard (Invitrogen Life Technologies) containing nine proteins ranging in size from 40 to 500 KDa for large protein analysis. The gel was stained with a ready-to-use Coomassie® G-250 stain following the SimplyBlue™ SafeStain Microwave Protocol (Invitrogen Life Technologies). The stained protein bands were analyzed using ChemiImager™ 440 v. 3.2.1 (Alpha Innotech Corp., San Leandro, Calif., U.S.A.) software.

Amino acid composition

Dry gelatin (1 g to 2 g) was weighed and hydrolyzed (200 μL, 0.1% phenol in 6 N HCl) at 110 °C for 24 h, dissolved in AE-Cys dilution buffer to a final volume of 10 mL and spundown. Fifty μL was injected into a Beckman 6300 amino acid analyzer (Beckman Instruments Inc., Fullerton, Calif., U.S.A.) and concentrations of amino acids (nmoles/injection) were obtained and mole percent was calculated.

Gel strength

Gel strength (Irish standard 1953) was determined on a 6.67% gel (w/w) by adding 7.5 g gelatin to 105 mL distilled water in a 250 mL flask, mixed with a magnetic bar stirrer using a PMC Dataplate® digital stirrer series 730 (Barnstead Intl., Dubuque, Iowa, U.S.A.) for 5 min at 500 rpm. The solution was heated in the flask covered with aluminum foil inside a water bath at 60 °C for 1 h with occasional stirring. The gelatin solution was degassed by vacuum and then 25 g solution was poured into 30 mL beakers, and thereafter refrigerated at 2 °C (maturation temperature) for 48 h. Dimensions of the sample were 3.3 cm in diameter and 6 cm in height. Gel strength was determined at 2 °C on an Instron model 5500R Universal Testing Machine (Instron Corp., Canton, Mass., U.S.A.) using a 100 N load cell with the cross-head speed at 10 mm/min and equipped with a 1.27-cm-dia flat-faced cylindrical stainless steel plunger. Measurements were taken as quickly as possible to reduce temperature change and experimental error. The maximum force (in N) was

recorded when the plunger penetrated 4 mm into the gelatin gels and there were four replications for each treatment.

pH

The pH of 6.67% (w/w) gelatin solutions at 25 °C was measured with a Beckman model 390 pH-meter (Beckman Instruments Inc.). Eight readings were made on each sample.

Gel clarity

Clarity was determined by measuring transmittance (%T) at 620 nm in a Varian spectrophotometer (Varian Inc., Scientific Instruments, Cary, N.C.) through 6.67% (w/w) gelatin solutions heated at 60 °C for 1 h. Eight readings were made on each sample.

Viscosity

Viscosity studies were determined in a Brookfield Digital Rheometer model DV−III+ with a TC-500 Refrigerated Bath/Circulator using a model 107 Programmable Temperature Controller running Rheocalc for Windows (Brookfield Engineering Laboratories Inc., Middleboro, Mass., U.S.A.). A small sample adapter along with spindle SC4–21 (0.66-mm dia, 1.23-mm long) was used to measure the gel set point temperature of the gelatin. For the experiments, 8.5 ± 0.1 g of 6.67% (w/w) gelatin solution was added to the small sample adapter. The initial testing temperature was 35 °C for mammalian gelatin and 25 °C for fish gelatin considering their differences in gelation temperature. Samples were allowed to rest in the small sample adapter for 30 min to equilibrate to the initial test temperature. The temperature was then lowered to 2 °C without noticeable ice crystal formation or until the gel set point temperature was reached. Gel set point temperature was indicated by a sharp rise in viscosity. The rate of temperature decline was 1 °C/min. Eight readings were made on each sample.

Gelatin film casting

Gelatin solutions (Irish standard 1953) were prepared by dissolving 7.5 g of gelatin in 105 mL distilled water (6.67% w/w) and mixed for 5 min at 500 rpm. These solutions were then heated in a water bath at 60 °C for 60 min, degassed and 20 mL was poured onto a flat Mylar film (Dow Chemical Co., Midland, Mich. U.S.A) on a glass plate. A stainless steel bar spreader with 0.06 or 0.08 mil gaps was used to obtain the same film thickness for mammalian and fish gelatins. The films were dried overnight at ambient temperature (25 °C) and peeled from the Mylar surface. Circular (65-mm dia) pieces were cut using a round watch glass and a razor blade. Film thickness was measured before testing water vapor permeability with a micrometer at five random positions by slowly reducing the micrometer gap until the first indication of contact.

Water vapor permeability of gelatin films

The ASTM E96–80 (ASTM 1989) "Water Method", modified to estimate the %RH at the film underside (McHugh and others 1993), was used to measure water vapor permeability with polymethylmethacrylate test cells (50.8-mm dia opening, 9.525-mm height). Eight 65-mm-dia gelatin films were mounted on the test cells filled with 6 mL distilled water and fixed with the top cover and a thin layer of silicone high vacuum grease (Dow Corning, Midland, Mich., U.S.A.) as sealant, by screwing tightly to avoid vapor leaks through cell joints.

Test cells were placed inside a cabinet containing anhydrous calcium sulfate (W.A. Hammond Drierite Co., Xenia, Ohio, U.S.A.) equipped with fans to provide high air movement to ensure 0 %RH throughout. The films equilibrated for an hour prior to starting the measurement of weight loss, which were done at least every 2 h and nine measurements were conducted in 2 d. The cabinet was inside an incubator at 25 °C.

By simple linear regression analysis the slope of weight loss compared with time was calculated to determine the water vapor transmission rate in g/h. Water vapor flux, permeance, and water vapor permeability were calculated according to values of test cell mouth area, %RH difference from the film underside, and mean film thickness, respectively (ASTM 1989; Avena-Bustillos and Krochta 1993; McHugh and others 1993). Eight replicates were made on each sample.

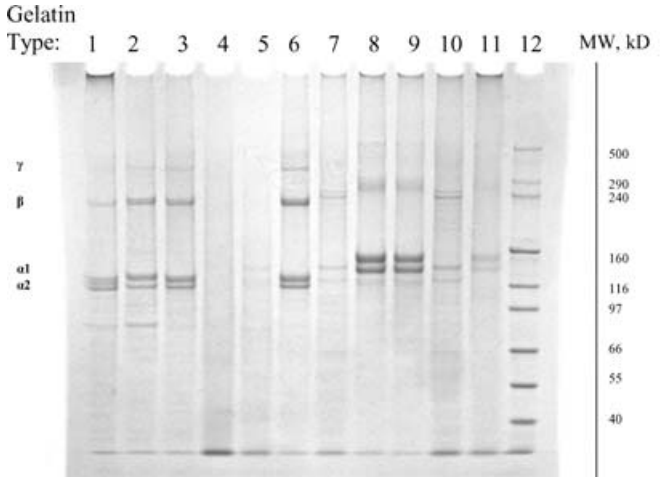
Statistical analyses

Data were analyzed by one-way analysis of variance and Tukey's multiple comparison tests at 95% confidence level using Minitab version 14.12.0 statistical software (Minitab Inc., State College, Penn., U.S.A.).

Results and Discussion

Molecular weight distribution of gelatin

Figure 1 shows the molecular weight distribution of the different gelatins determined from SDS gel electrophoresis. Both pollock and salmon gelatins as well as commercial cold-water fish gelatins showed higher band mobility than catfish gelatin and mammalian gelatins, as an indication of differences in protein hydrophobicity.



1. Pollock
2. Salmon
3. HMW CWF
4. HYD CWF
5. WWF catfish
6. CWF
7. Pork skin 1
8. ND cattle bone
9. D cattle bone
10. Pork skin 2
11. Cattle hide
12. Molecular weight marker

Figure 1—SDS gel electrophoresis molecular weight distribution of mammalian, warm- and cold-water fish skin gelatins

Fish and mammalian gelatins have a broad but a distinctive molecular weight distribution related to the collagen structure and production process, which includes independent single α_1- and α_2-chains; one α_1- and one α_2-chain covalently cross-linked to form β_{12}-chains or two α_1-chains to form β_{11}-chains (Balian and Bowes 1977). Also, two covalently cross-linked α_1-chains and one α_2-chain form γ_{112}-chains. Gudmundsson (2002) and Balian and Bowes (1977) reported a third α chain, α_3, isolated from codfish collagen, differing very slightly from α_1-chains. Pollock gelatin also showed an α_3-chain as indicated in Figure 1. Pollock and cod share the same aquatic environment.

β-chain and γ-chain aggregations were present in salmon and pollock skin gelatins as well as in commercial mammalian and fish skin gelatins. Large amounts of β- and γ-chains have shown to negatively affect some functional properties of gelatins (Muyonga and others 2004). As expected, the SDS-PAGE of hydrolyzed gelatin did not show bands as an indication of absence of intact gelatin chains and large amounts of low molecular weight peptides.

The α_1/α_2-chain density ratio ranged from 1.2 to 1.8 in this study for all gelatin samples, except hydrolyzed cold-water fish gelatin, as both α chains were broken down during hydrolysis. Gomez-Guillen and others (2002) reported that an α_1/α_2-chain density ratio of less than one could indicate damage or partial loss of α-chains during the extraction procedure. In Type I collagen this ratio is typically 2.0 if β- and γ-chains are absent. In this study, relative amounts of α_1-chains in fish gelatins were in the range of 30% to 35% similar to data reported by Gomez-Guillen and others (2002) and lower than in mammalian gelatins (31% to 44% range).

The hydrophilic nature of gelatins made it not possible to determine the exact molecular weight of the different gelatin chain bands by comparing with the SDS-PAGE standard proteins used in this study.

Amino acid composition

The composition of gelatin is similar to that of the collagen from which it has been prepared. However, alkali-processed gelatins (Type B) have higher hydroxyproline and lower tyrosine contents than either acid-processed (Type A) gelatins or the original collagen (Eastoe and Leach 1977). As shown in Table 2, cold-water fish gelatins have significantly ($P < 0.01$) less hydroxyproline, proline, valine, and leucine than mammalian gelatins. Otherwise, cold-water fish gelatins have significantly ($P < 0.01$) more glycine, serine, threonine, aspartic acid, methionine, and hystidine than mammalian gelatins. Cold-water fish and mammalian gelatins have the same proportion of alanine, glutamic acid, cysteine, isoleucine, tyrosine, phenylalanine, homocysteine, hydroxylysine, lysine, and arginine. In spite of differences in amino acid compositions, all types of gelatins have an almost constant hydroxyl amino acid content (Eastoe and Leach 1977). All amino acids commonly found in proteins occur in gelatin with the probable exception of tryptophan and cystine, which are thought to result from keratins and other protein contaminants (Eastoe and Leach 1977).

Catfish gelatin has an intermediate amino acid composition compared to mammalian and cold-water fish gelatins. In general, warm-water fish gelatins have higher concentrations of proline and hydroxyproline than cold-water fish gelatins, but less than mammalian gelatins (Eastoe and Leach 1977). The proline and hydroxyproline contents are approximately 30% for mammalian gelatins, 22% to 25% for warm-water fish gelatins (Tilapia and Nile perch), and 17% for cold-water fish gelatin (cod) (Muyonga and others 2004). Proline and hydroxyproline stabilize the ordered triple helical conformation when renatured gelatin forms a gel network (Gomez-Guillen and others 2002; Haug and others 2004). In particular, hydroxyproline is believed to play an important role in the stabilization of the triple-stranded collagen helix due to hydrogen bonding to its hydroxyl group. The higher content of proline, hydroxyproline, and alanine in mammalian gelatins has been reported as one of the major reasons for higher viscosity properties when compared to fish gelatins (Sarabia and others 2000).

pH and clarity

As shown in Table 3, lab-extracted pollock and salmon skin gelatin solutions at 6.67% (w/w) had significantly lower pH values than commercial cold-water fish gelatin solutions at the same concentrations. Although there were differences in the pH of cold-water fish gelatin solutions, other properties, including gel strength, gel set temperature, and water vapor permeability of the resulting gelatin films, were generally similar. Stainsby (1977) indicated that small variations of pH could give rise to quite marked changes in intrinsic viscosity, especially at low ionic strength (0.2% gelatin concentration).

Table 2—Amino acid composition (% mole) of cold-water, warm-water fish skin and mammalian gelatins

Amino acid	Pollock	Salmon	HMW CWF	HYD CWF	CWF	WWF catfish	Pork skin 1	Pork skin 2	Cattle hide	ND cattle bone	D cattle bone
Alanine	10.88	12.49	10.87	11.43	11.04	12.40	11.39	11.06	11.91	11.64	11.48
Arginine	5.18	5.06	5.20	5.20	5.21	5.00	5.19	5.08	4.98	4.86	4.78
Aspartic acid	5.21	5.12	5.11	5.09	5.12	4.59	4.63	4.66	4.83	4.42	4.36
Cystine	0.14	0.08	0	0.08	0.26	0.10	0.16	0.09	0.04	0.10	0
Glutamic acid	7.17	7.25	7.21	7.12	7.11	7.22	7.27	7.44	7.78	7.13	7.18
Glycine	35.74	35.54	35.93	35.40	36.14	34.01	32.34	31.73	28.74	34.22	33.53
Histidine	0.80	0.87	0.75	0.62	0.74	0.60	0.48	0.50	0.47	0.41	0.39
Homocystine	0.16	0.12	0.13	0.04	0.05	0.09	0.02	0.03	0.02	0.04	0.06
Hydroxylysine	0.61	0.76	0.64	0.59	0.61	0.58	0.68	0.70	0.86	0.75	0.84
Hydroxyproline	5.30	5.56	5.48	5.47	4.93	7.72	8.53	9.78	10.39	9.49	10.42
Isoleucine	1.07	0.97	1.07	0.97	1.07	1.17	1.01	1.05	1.28	1.16	1.11
Leucine	2.10	1.83	1.99	2.09	2.02	2.09	2.58	2.50	2.59	2.54	2.47
Lysine	2.78	2.47	2.72	2.68	2.75	3.10	2.83	2.80	2.77	2.65	2.61
Methionine	1.13	1.00	1.03	1.46	1.25	0.49	0.54	0.29	0.33	0.49	0.24
Phenylalanine	1.20	1.27	1.19	1.35	1.25	1.30	1.44	1.37	1.33	1.25	1.28
Proline	10.09	10.79	9.98	10.42	10.12	11.14	13.47	13.12	13.78	12.11	12.12
Serine	5.85	4.73	6.11	5.89	5.76	3.61	3.07	3.39	3.52	2.91	3.13
Threonine	2.68	2.55	2.62	2.30	2.54	2.60	1.69	1.87	2.01	1.67	1.87
Tyrosine	0.24	0.13	0.24	0.15	0.21	0	0.39	0.37	0.22	0	0
Valine	1.67	1.41	1.74	1.66	1.81	2.19	2.30	2.18	2.16	2.14	2.12

Table 3—pH, transmittance (%T) and gel set temperature of 6.67% solutions of cold-water, warm-water fish skin and mammalian gelatins

Gelatin type	pH	% T	Gel set temperature (°C)
Pollock	3.25 ± 0.13 (a)	92.59 ± 0.62 (d)	4.58 ± 0.63 (a)
Salmon	3.24 ± 0.04 (a)	44.00 ± 0.30 (b)	5.33 ± 0.29 (a)
HMW CWF	5.59 ± 0.09 (d)	94.87 ± 0.06 (d)	5.55 ± 0.21 (a)
HYD CWF	4.93 ± 0.08 (c)	95.30 ± 0.44 (d)	No[a]
CWF	5.82 ± 0.06 (d)	90.90 ± 0.10 (d)	5.23 ± 0.40 (a)
WWF Catfish	5.57 ± 0.17 (d)	1.27 ± 0.01 (a)	11.90 ± 0.28 (b)
Pork skin 1	5.21 ± 0.09 (c)	93.30 ± 0.36 (d)	24.38 ± 0.08 (c)
Pork skin 2	4.62 ± 0.31 (b)	86.87 ± 0.81 (d)	24.45 ± 0.35 (c)
Cattle hide	5.01 ± 0.24 (c)	75.50 ± 0.44 (c)	23.15 ± 0.07 (c)
ND cattle bone	5.57 ± 0.15 (d)	95.90 ± 0.01 (d)	24.06 ± 0.05 (c)
D cattle bone	5.75 ± 0.09 (d)	98.97 ± 0.81 (e)	22.85 ± 0.01 (c)

[a]Not detectable in the range 25°C–2°C.
*Different letters within a column indicated significant difference at $P < 0.05$.

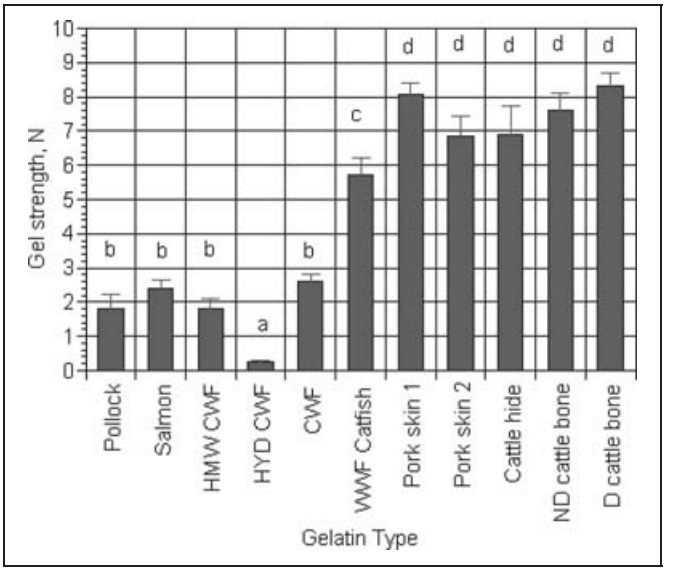


Figure 2—Gel strength of 6.67% solution gels of cold-water, warm-water fish skin and mammalian gelatins

As indicated in Table 3, commercial catfish gelatin and lab-extracted salmon gelatin solutions were very opaque with low %T values, while deionized cattle bone gelatin solution was practically clear. Turbidity and dark color of gelatin is commonly caused by inorganic, protein, and mucosubstance contaminants, introduced or not removed during its manufacture (Eastoe and Leach 1977).

Viscosity

Mammalian gelatins had much higher gel set temperatures than warm-water and cold-water fish skin gelatins, as shown in Table 3. Mammalian gelatins exhibited a large increase in viscosity at approximately 24 °C, whereas cold-water fish gelatins showed large increases in viscosity at 5 °C. This large viscosity increase resulted from changes in the gelatin molecule's conformation during the cooling process. At higher temperatures, the gelatin molecules behave as random coils in solution and all gelatins had low viscosities. As the temperature is lowered, the gelatin molecules begin to form triple helical junction zones and partly revert to the collagen structure. As more physical cross-linking occurs, some network structure eventually develops and the viscosity rapidly increases in value. In addition, gelatin samples with larger elastic modulus values, also indicated by higher gelation temperatures, contained higher concentrations of helical structures (Gomez-Guillen and others 2002; Joly-Duhamel and others 2002; Simon and others 2003). As shown in Table 3, porcine gelatin gelled at the highest temperature of any of the gelatins tested, suggesting that porcine gelatin had a higher concentration of helical structures than other gelatins.

The difference in gel thermostability between mammalian and fish skin gelatins can be attributed to differences in content of proline and hydroxyproline. The lower content of these two amino acids (Table 2) probably gives fish gelatins their lower gel set temperatures (Haug and others 2004). Porcine gelatin contains 22.9 mol% proline and hydroxyproline, whereas pollock and salmon gelatins contain 15.4 and 16.4 mol% proline and hydroxyproline, respectively. A higher proline and hydroxyproline concentration has been shown to result in higher gelation temperatures, which is consistent with results shown in Table 3. The influence of molecular weight distribution on gel set temperature for the different types of gelatin was not evident except for hydrolyzed gelatin, which failed to gel. Lower content of high molecular weight fractions (β- and γ-chains) for bone gelatins has been associated with lower viscosity, melting, and setting point and longer setting time (Muyonga and others 2004).

Gel strength

Gelatin is a biological polymer that forms thermoreversible gels. Even after formation, the structure of the gel is not static and continues to evolve and change because of the instability of low energy interactions within the gel network (Tosh and others 2003). As shown in Figure 2, cold-water fish skin gelatins had lower gel strength than warm-water catfish gelatins. Mammalian gelatins had the highest gel strengths, which varied within species and gelatin type (extracted under acidic, Type A, or basic conditions, Type B) from 6.8 N to 8.3 N. As expected, hydrolyzed cold-water fish gelatin had the lowest gel strength.

Water vapor permeability

Hydrophilic films, such as gelatin films, exhibit positive slope relationships between thickness and water vapor permeability resulting from variations in the water vapor partial pressure at the underside of films during testing (McHugh and others 1993); therefore it is essential for practical comparison purposes of water vapor permeability values to get similar film thicknesses. There was no statistical difference between film thicknesses among the different gelatins tested. Mean and pooled standard deviation thicknesses of gelatin films were 0.0496 mm and 0.0041 mm, respectively. The average relative humidity at the gelatin films underside was estimated as 80 %RH for these experiments using the water vapor correction method (McHugh and others 1993).

Hydrophilic edible films are very prone to plasticization from water, which tends to cluster within the polymer matrix. It is recognized that an increase in plasticizer is directly proportional to an increase in water vapor permeability (McHugh and others 1993; Arvanitoyannis and others 1998a, 1998b; Carvalho and Grosso 2004).

As shown in Figure 3, water vapor permeabilities (WVP) of the five cold-water fish gelatin films studied were not significantly different and averaged 0.932 gmm/m^{2}hkPa. Cold-water fish gelatin films were significantly lower in WVP than warm-water fish and mammalian gelatin films (1.309 and 1.884 gmm/m^{2}hkPa, respectively) at 25 °C, and 0/80 %RH differential through films with the same thickness. The average WVP of the five mammalian gelatin films were not significantly different, but were higher than the warm-water fish gelatin film. The low water vapor permeability of cold-water fish gelatins,

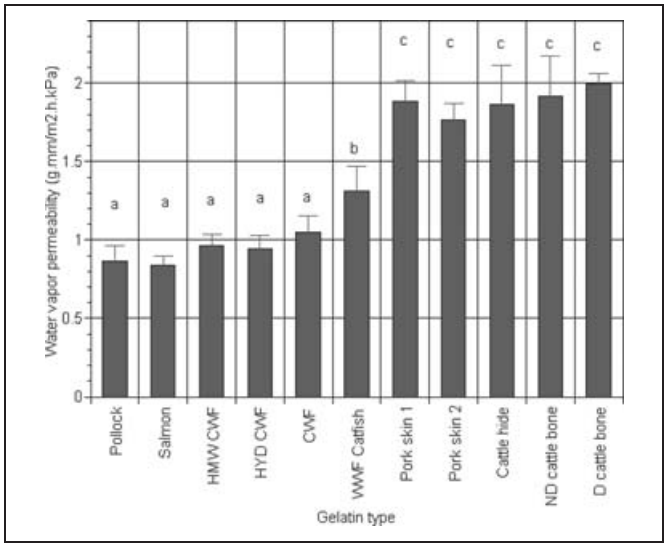


Figure 3—Water vapor permeability of films from cold-water, warm-water fish skin and mammalian gelatins

and to a lesser degree warm-water fish gelatin, is related to their high hydrophobicity, due to reduced amounts of proline and hydroxyproline compared to mammalian gelatins. A lower water permeability of gelatin films could be related to the low temperature (20 °C) used for casting the aqueous gelatin solutions. This casting method has been shown to enhance development of a higher percentage of renaturation (crystallinity) of gelatin, which also could result in a decrease by one or two magnitude orders of CO_2 and O_2 permeability of gelatin films (Arvanitoyannis and others 1998b).

Conclusion

This study demonstrated significant differences in physical and chemical properties between mammalian, warm- and cold-water fish gelatins. Lower water vapor permeability of warm-water fish gelatin, and particularly of cold-water fish gelatin films can be useful for applications related to reduce water loss in gel-encapsulated drugs and in refrigerated and frozen food systems.

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